

CLAIMS

What is claimed is:

1. A method of detecting chitinous material in a processed non-chitinous biological sample, said method comprising
contacting said biological sample with a probe that is a lectin that binds chitin;
contacting said biological sample with a pectinase; and
detecting binding of said lectin to a chitin wherein said binding indicates the presence of chitin in said biological sample.

10 2. The method of ~~claim 1~~, wherein said chitin comprises an insect, an insect part, or any animal of the phylum Arthropoda subphylum Crustacea.

3. The method of ~~claim 1~~, wherein said chitin is a component of a micro-organism.

15 4. The method of ~~claim 3~~, wherein said microorganism is selected from the group consisting of a fungus, a mold, and a yeast.

5. The method of ~~claim 3~~, wherein said microorganism is a selected from the group consisting of a fungus of phylum Ascomycota, a fungus of phylum Basidiomycota, a fungus of phylum Chytridiomycota, a fungus of phylum Zygomycota, and a member of the phylum Oomycota in the Stramenopila kingdom.

20 6. The method of ~~claim 3~~, wherein said microorganism is a fungus selected from the group consisting of *Cladosporium spp*, *Fusarium spp*, *Stemphylium spp*, *Alternaria spp*, *Geotrichum spp*, *Fusarium spp*, *Rhizopus spp*, *Botrytis spp*, *Phytophthora spp*, *Pythium spp*, and *Pythium spp*.

25 7. The method of ~~claim 1~~, wherein said biological sample is a selected from the group consisting of an agricultural product, a food product, a wood product, a textile, and an animal tissue product.

8. The method of ~~claim 7~~, wherein said agricultural product is selected from the group consisting of a fruit, a vegetable, a grain, forage, a silage, a juice, a wood, a flower, and a seed.

5 9. The method of ~~claim 7~~, wherein said agricultural product is a fruit selected from the group consisting of a tomato, a pepper, a grape, an apple, an orange, a lemon, a berry.

10 10. The method of ~~claim 1~~, wherein said lectin is selected from the group consisting of wheat germ agglutinin (WGA), succinylated WGA, pokeweed lectin, tomato lectin, potato lectin barley lectin, rice lectin, stinging nettle lectin, a vicilin, a chitovibrin, a Vibrio lectin, and a hevein.

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11. The method of ~~claim 1~~, wherein said method further comprises contacting said sample with a blocking reagent.

12. The method of ~~claim 11~~, method wherein said blocking reagent is serum albumin.

15 13. The method of ~~claim 1~~, wherein said detecting comprises filtering said sample.

14. The method of ~~claim 13~~, wherein said detecting comprises eluting bound lectin.

20 15. The method of ~~claim 14~~, wherein said eluting comprises contacting said lectin with a chitin, a chitin degradation product or a chitin analogue.

16. ~~The method of claim 15, wherein said chitin degradation product is N-acetyl D-glucosamine.~~

17. The method of ~~claim 1~~, wherein said lectin is labeled with a detectable label.

18. The method of claim 17, wherein said label is selected from the group consisting of a radioactive label, a magnetic label, a colorimetric label, an enzymatic label, a fluorescent label, a metal, an antibody, a biotin, and an avidin or streptavidin.

19. The method of claim 17, wherein said label is a fluorescent label.

5 20. The method of claim 19, wherein said detecting comprises using a fluorometer to detect fluorescence of said label.

10 21. The method of claim 20, wherein said detecting comprises:
filtering said sample;
washing said filter to remove unbound chitin;
eluting bound lectin with a chitin, a chitin degradation product or a
chitin analogue; and
detecting said eluted lectin with said fluorometer.

22. The method of claim 20, wherein said fluorometer uses a bandpass filter.

15 23. The method of claim 20, wherein said fluorometer is a surface-reading fluorometer.

24. The method of claim 1, wherein said method is performed at a pH greater than about pH 7.

20 25. The method of claim 1, wherein said method is performed at about pH 8.

26. The method of claim 1, wherein said pectinase comprises an enzyme selected from the group consisting of polygalacturonases, pectinesterases, pectin lyases, and hemicellulases.

25 27. The method of claim 1, wherein the processed biological sample is a sample that has been subjected to an operation selected from the group consisting of comminuting, homogenizing, heating, evaporation, lyophilization, filtering, concentrating, filtering, fermenting, freezing, and blanching.

28. The method of ~~claim 1~~, wherein
the biological sample is selected from the group consisting of a fruit, a
vegetable, a fruit juice, and a vegetable juice;

5 said lectin is a fluorescently labeled lectin selected from the group
consisting of wheat germ agglutinin (WGA), succinylated WGA, pokeweed lectin, tomato
lectin, potato lectin, barley lectin, rice lectin, stinging nettle lectin, a vicilin, a chitovibrin, a
Vibrio lectin, and a hevein;

10 said pectinase is a pectinase selected from the group consisting of
polygalacturonases, pectinesterases, pectin lyases and hemicellulases;

said sample is processed by comminuting, homogenizing, heating,
evaporation, lyophilization, filtering, concentrating, filtering, fermenting, freezing, and
blanching; and

said detecting comprises detecting a signal from the fluorescent label
labeling said lectin.

15 29. A method of detecting chitinous material in a non-chitinous biological
sample, said method comprising
in a solution at a pH ranging from about pH 7 to about pH 9
contacting said biological sample with a fluorescently labeled probe that is a lectin that binds
chitin; and
20 detecting binding of said lectin to a chitin wherein said binding
indicates the presence of chitin in said biological sample.

30. The method of ~~claim 29~~, wherein said chitin comprises an insect or
insect part.

25 31. The method of ~~claim 29~~, wherein said chitin is a component of a
micro-organism.

32. The method of ~~claim 31~~, wherein said microorganism is selected from
the group consisting of a fungus, a mold, and a yeast.

33. The method of ~~claim 31~~, wherein said microorganism is a fungus
selected from the group consisting of *Cladosporium herbarum*, *Fusarium oxysporum*, and

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Stemphylium botryosum, *Alternaria alternata*, *Geotrichum candidum*, *Fusarium oxysporum*,
Rhizopus stolonifer, *Botrytis cinerea*, *Phytophthora parasitica*, *Pythium aphanidermatum*,
Pythium ultimum.

5 34. The method of claim 29, wherein said biological sample is selected from the group consisting of an agricultural product, a food product, a wood product, a textile, and an animal tissue product.

35. The method of claim 34, wherein said agricultural product is selected from the group consisting of a fruit, a vegetable, a grain, forage, a silage, a juice, a wood, a flower, and a seed.

10 36. The method of claim 34, wherein said agricultural product is a fruit selected from the group consisting of a fruit, a vegetable, a grain, forage, a silage, a juice, a wood, a flower, and a seed.

15 37. The method of claim 29, wherein said lectin is selected from the group consisting of wheat germ agglutinin (WGA), succinylated WGA, pokeweed lectin, tomato lectin, potato lectin, barley lectin, rice lectin, stinging nettle lectin, a vicilin, a chitovibrin, a Vibrio lectin, and a hevein.

38. The method of claim 29, wherein said method further comprises contacting said sample with a blocking reagent.

20 39. The method of claim 38, wherein said blocking reagent is serum albumin.

40. The method of claim 29, wherein said detecting comprises filtering said sample.

41. The method of claim 40, wherein said detecting comprises eluting bound lectin.

25 42. The method of claim 41, wherein said eluting comprises contacting said lectin with a chitin, a chitin degradation product or a chitin analogue.

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43. The method of claim 42, wherein said chitin degradation product is N-acetyl D-glucosamine.

44. The method of claim 29, wherein said lectin is labeled with a detectable label.

5 45. The method of claim 17, wherein said label is selected from the group consisting of a radioactive label, a magnetic label, a colorimetric label, an enzymatic label, a fluorescent label, a metal, an antibody, a biotin, and an avidin or streptavidin.

46. The method of claim 17, wherein said label is a fluorescent label.

10 47. The method of claim 19, wherein said detecting comprises using a fluorometer to detect fluorescence of said label.

15 48. The method of claim 20, wherein said detecting comprises:
filtering said sample;
washing said filter to remove unbound chitin;
eluting bound lectin with a chitin, a chitin degradation product or a chitin analogue; and detecting said eluted lectin with said fluorometer.

49. The method of claim 20, wherein said fluorometer is a surface-reading fluorometer.

50. The method of claim 29, wherein said method is performed at a basic pH greater than about pH 7.5.

20 51. The method of claim 29, wherein said method is performed at a basic pH about pH 8.0.

25 52. The method of claim 29, wherein
the biological sample is selected from the group consisting of a fruit, a vegetable, a fruit juice, and a vegetable juice;
said lectin is a fluorescently labeled lectin selected from the group consisting of wheat germ agglutinin (WGA), succinylated WGA, pokeweed lectin, tomato

lectin, potato lectin barley lectin, rice lectin, stinging nettle lectin, a vicilin, a chitovibrin, a Vibrio lectin, and a hevein; and

said detecting comprises detecting a signal from the fluorescent label labeling said lectin.

5 53. The method of claim 29, further comprising contacting said biological sample with a pectinase.

54. The method of claim 53, wherein said pectinase is selected from the group consisting of polygalacturonases, pectinesterases, pectin lyases and hemicellulases.

10 55. In a biological sample, a lectin that specifically binds to chitin, wherein said lectin is bound to a chitinous contaminant of said sample, and said lectin is labeled with a label that provides a signal distinguishable from a background signal where said signal indicates the presence or quantity of chitinous contaminant in said biological sample.

15 56. The biological sample of claim 55 wherein the pH of said sample is basic ranging from about pH 7 to about pH 9.

57. The biological sample of claim 55, wherein said sample is a processed sample.

58. The biological sample of claim 55, wherein said biological sample is an agricultural product.

20 59. The biological sample of claim 55, wherein said agricultural product is selected from the group consisting of consisting of a fruit, a vegetable, a grain, forage, a silage, a juice, a wood, a flower, and a seed.

25 60. The biological sample of claim 55, wherein said agricultural product is a fruit selected from the group consisting of a tomato, a pepper, a grape, an apple, an orange, a lemon, a berry.

61. The biological sample of claim 55, wherein said sample further comprises an exogenously supplied pectinase.

62. The biological sample of claim 55, wherein said lectin is labeled with a label selected from the group consisting of a radioactive label, a magnetic label, a colorimetric label, an enzymatic label, a fluorescent label, a metal, an antibody, a biotin, and an avidin or streptavidin.

5 63. A kit for detecting chitinous material in a non-chitinous biological sample, said kit comprising:
a first container containing a lectin that specifically binds a chitinous material;
a second container containing a pectinase.

10 64. The kit of claim 63, wherein said first container and said second container are the same container.

65. The kit of claim 63, wherein said pectinase is selected from the group consisting of polygalacturonases, pectinesterases, pectin lyases and hemicellulases.

15 66. The kit of claim 63, wherein said lectin is selected from the group consisting of wheat germ agglutinin (WGA), succinylated WGA, pokeweed lectin, tomato lectin, potato lectin, barley lectin, rice lectin, stinging nettle lectin, a vicilin, a chitovibrin, a *Vibrio* lectin, and a hevein.

67. The kit of claim 63, further comprising a label for labeling said lectin.

68. The kit of claim 63, wherein said lectin is labeled.

20 69. The kit of claim 63, wherein said lectin is labeled with a label selected from the group consisting of a radioactive label, a magnetic label, a colorimetric label, an enzymatic label, a fluorescent label, a metal, an antibody, a biotin, and an avidin or streptavidin.

25 70. The kit of claim 63, further comprising a transparent centrifugable receptacle suitable for use with a surface-reading fluorometer.

71. The kit of claim 63, further comprising a bandpass filter for that passes light emitted by a fluorescent label in said kit.

72. A method of detecting a fluorochrome bound to one phase of a two-phase mixture, said method comprising
contacting a transparent surface of a receptacle with a solid or semi-solid phase of said two phase mixture;
5 illuminating said solid or semisolid phase of said two phase mixture through said transparent surface; and
detecting through said transparent surface a fluorochrome bound to said solid or semi-solid phase of said two-phase mixture.

73. The method of claim 72, wherein said receptacle is a centrifuge tube.

10 74. The method of claim 72, wherein said receptacle is a flow-through centrifuge.

75. The method of claim 73 wherein said contacting comprises spinning said receptacle so that the solid or semi-solid phase is deposited against the transparent surfaced.

15 76. The method of claim 73 wherein said two-phase mixture comprises a biological sample.

77. The method of claim 73 wherein said fluorochrome is a chitin-specific fluorescently labeled lectin.

20 78. A surface-reading fluorometer comprising:
a receptacle having a transparent surface, said receptacle being compatible with centrifugation in a centrifuge;
a light source for illuminating a sample through said transparent surface; and
25 a detector disposed to detect fluorescence through said transparent surface.